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Diethylcarbamazine Citrate, an Antifilarial Drug, Stimulates Human Granulocyte Adherence

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Incubation with diethylcarbamazine citrate caused significant augmentation of human neutrophil and eosinophil adherence to tissue culture plastic. This effect was dose dependent and cell dependent, with eosinophils showing greater sensitivity and a greater adhesive response to the drug than did neutrophils. Eosinophils preincubated with diethylcarbamazine citrate demonstrated decreased adhesive responses to other adherence-augmenting stimuli. Use of Fc-treated plastic augmented diethylcarbamazine citrate-stimulated neutrophil (but not eosinophil) adherence. Direct stimulation of host effector cell adherence may explain, in part, the therapeutic action of diethylcarbamazine citrate in vivo.

The antifilarial drug diethylcarbamazine citrate (DEC) has been in clinical use for almost 35 years, yet its pharmacological mechanism of action remains obscure. Although DEC has no direct microfilaricidal activity against most species, the drug can be shown to enhance host effector cell destruction of microfilariae both in vitro (3, 8) and in vivo (7, 8, 18, 20, 21). DEC causes rapid clearance of circulating microfilariae in animals with either active or passive humoral immunity (7, 8, 21). When cleared from the bloodstream, DEC-treated microfilariae lodge in the liver, spleen, and kidney, where they are rapidly surrounded by host inflammatory cells, including granulocytes (7, 8, 15, 18, 20, 21). In vitro study shows that DEC enhances leukocyte adherence to microfilariae in the presence of immune serum (3, 9, 14). Controversy remains, however, as to whether this enhancement of cell attachment is a drug-mediated effect on the cell, on the parasite surface, or on both. By using a model of granulocyte attachment to tissue culture plastic, our study examined the direct effect of DEC on neutrophil and eosinophil adhesive responses.

(Portions of this work have appeared previously [C. H. King, B. M. Greene, and P. J. Spagnuolo, Clin. Res. 30:516A, 1982.])

Normal granulocytes were separated via discontinuous metrizamide gradients (19), yielding neutrophils of 97 to 100% purity and eosinophils of 70 to 95% purity. Adherence to polystyrene wells was studied using a modification of a previously described method (4). Briefly, granulocytes (1.5×10^5) were incubated in plastic microtiter wells (Costar, Cambridge, Mass.) for 1 h at 37°C with Eagle minimal essential medium (K. C. Biologicals, Lenexa, Kans.) containing 10% heat-inactivated normal human serum, with or without chemotactic factors. Chemotactic factors included 1 μM *N*-formylmethionylleucyl-phenylalanine (FMLP; Sigma Chemical Co., St. Louis, Mo.) and 10% zymosan-activated serum (ZAS; prepared by incubation of 5 mg of zymosan [Sigma] per ml of fresh normal human serum at 37°C for 30 min). DEC (American Cyanamid Co., Pearl River, N.Y.) was prepared by dissolving the drug in medium and adjusting the pH to 7.2 to 7.4. After the 1-h incubation, the plastic wells were washed three times with Hanks balanced salt solution (K. C. Biologicals), and adherent cells were quantitated with a particle counter (Coulter Electronics Inc., Hialeah, Fla.). Base-line adherence and stimulated adherence were lower overall for eosinophils (base line, 2 to 7%; stimulated, 10 to 25%) than for neutrophils (base line, 5 to 10%; stimulated, up to 70%). Results for each experiment were expressed as a mean stimulation index (SI) (SI = experimental adherence/base-line adherence × 100) calculated for each experimental group. Significance of results was determined by using Student's *t* test.

The first series of 10 experiments established the effect of various concentrations of DEC (1 to 10 μg/ml) on eosinophil and neutrophil adherence (Fig. 1). The adhesive response was concentration dependent, with a peak effect on eosinophil adherence at 5 μg/ml (12.7 μM) (SI = 187 ± 32; $P < 0.01$), whereas the greatest effect on neutrophils was seen at 10 μg/ml (25.6 μM)

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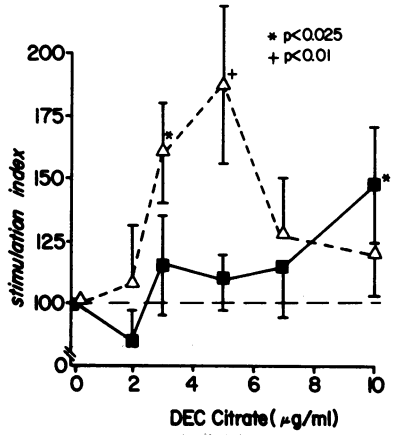

FIG. 1. Effect of DEC on human granulocyte adherence to plastic. DEC was incubated with purified human neutrophils and eosinophils. Significant enhancement of eosinophil adherence (△) occurred at 3 μg/ml (SI = 160 ± 21 [$P < 0.025$]) and 5 μg/ml (SI = 187 ± 32 [$P < 0.01$]). Significant enhancement of neutrophil adherence (■) (SI = 138 ± 16 [$P < 0.025$]) occurred at 10 μg/ml.

(SI = 147 ± 23; $P < 0.025$). For comparison, a similar stimulation of adherence was seen with 1 μM FMLP (SI of eosinophils = 194 ± 22 [$P < 0.0025$]; SI of neutrophils = 713 ± 134 [$P < 0.0005$]) and with 10% ZAS (SI of eosinophils = 283 ± 56 [$P < 0.0025$]; SI of neutrophils = 784 ± 137 [$P < 0.0005$]) in parallel experiments. The augmentation of adherence seen with DEC was not due to an effect of the citrate anion, since equimolar concentrations of sodium citrate did not stimulate granulocyte adherence (data not shown).

To assess whether the action of DEC was related to the effects of other known granulocyte stimuli (1, 12), cells were preincubated with DEC and then washed before rechallenge with ZAS or FMLP. Figure 2 demonstrates the effect of DEC preincubation (10 μg/ml for 15 min at 37°C) on eosinophil and neutrophil adhesive responses to these agents. As noted, DEC preincubation significantly inhibited the eosinophil response to ZAS and FMLP but had no effect on the neutrophil response. This effect was not seen at a lower DEC concentration (5 μg/ml).

The effect of DEC on immunoglobulin-mediated adherence was examined by using plastic wells pretreated with human Fc fragments (6.8 μg/cm^2 of well surface; Cappel Laboratories, Downington, Pa.). Wells were preincubated with Fc for 1 h at 37°C, washed three times with buffer, and then used in adherence studies. Incubation with ^{125}I-labeled Fc tracer revealed that ca. 17% of Fc fragments remained bound to the wells after washing. Cell adherence to Fc-treated wells was compared with adherence to control wells which had been preincubated with Fab fragments or with buffer alone. Use of Fc-treated wells did not significantly increase baseline adherence for either eosinophils or neutrophils. Neutrophils stimulated with either 1 μM FMLP or 10 μg of DEC per ml, however, showed significantly greater adherence to Fc-treated wells than to Fab-treated or untreated wells. For neutrophils in DEC, adherence increased by 88 ± 15% ($P < 0.025$); for neutrophils in FMLP, adherence increased by 26 ± 6% ($P < 0.025$). This DEC-mediated enhancement of adherence to Fc-treated plastic was not seen with eosinophils.

In separate experiments, neutrophil chemotaxis and eosinophil chemotaxis were studied under agarose (11) by using cells preincubated with and without DEC (5 to 40 μg/ml). DEC did not inhibit the migratory response to FMLP, and DEC itself (1 to 400 μg/ml) was not chemotactic (data not shown). Study of neutrophil aggregation (16) with a dual-channel aggregometer (Payton Associates, Buffalo, N.Y.) showed that DEC (5 to 400 μg/ml) did not induce aggregation, nor could it inhibit the aggregatory response to FMLP or ZAS (data not shown).

Although preliminary, these data highlight significant differences between eosinophil and neutrophil adhesive responses to DEC. DEC at therapeutic concentrations (3 to 5 μg/ml) specifically augmented eosinophil adherence but not neutrophil adherence. Neutrophil adherence

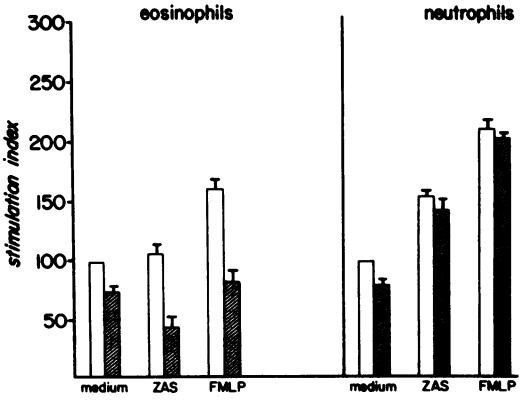

FIG. 2. Adherence of human granulocytes to plastic: effect of DEC preincubation on ZAS- and FMLP-stimulated adherence. Normal human granulocytes prepared via metrizamide fractionation were preincubated with DEC (10 μg/ml [▨]) or medium (□) for 15 min at 37°C, washed in minimal essential medium, and then added to various wells containing medium, 10% ZAS, or 10^{-6} M FMLP. DEC preincubation significantly ($P < 0.0025$) inhibited the eosinophil response to ZAS (ΔSI = −55 ± 6, three experiments) and FMLP (ΔSI = −78 ± 6, three experiments) without affecting the neutrophil response.

could be augmented in the presence of higher concentrations of DEC (10 μg/ml), particularly if Fc-coated plastic was used as a substrate. In contrast to neutrophils, eosinophil adherence to Fc-coated plastic was limited, perhaps reflecting that these cells have fewer surface immunoglobulin receptors than do neutrophils (13).

Preincubation of granulocytes with DEC reduced the eosinophil, but not neutrophil, response to subsequent cell stimulation with chemotactic factors. These data suggest that DEC may deactivate the eosinophil adhesive response to subsequent FMLP and ZAS stimulation and imply that DEC stimulation of adherence may occur via mechanisms similar to those for FMLP and ZAS. Alternatively, the inhibition of the eosinophil adhesive response (by preincubation with DEC) may reflect exhaustion of biochemical components necessary for subsequent stimulation with chemotactic factors. The mechanisms of DEC stimulation of cell adhesiveness are unclear. DEC has been shown to inhibit the synthesis of leukotrienes B_4 and C_4 in rat mastocytoma cells (10). DEC may shunt the metabolism of arachidonic acid in granulocytes to other biologically active prostanoids such as prostaglandin D_2, 5-monohydroxyeicosatetraenoic acid, or thromboxane A_2. 5-Monohydroxyeicosatetraenoic acid is a known promoter of eosinophil adherence to complement (but not immunoglobulin G)-opsonized erythrocytes and is a promoter of eosinophil chemokinesis (6). In addition, thromboxane A_2 has been implicated as a potential mediator of complement-induced neutrophil adherence to plastic (17). Recent research indicates that degranulation augments granulocyte adherence. In particular, release of lactoferrin by neutrophils promotes cell adherence to substrates and to other cells (2). It is possible that DEC may induce granule release to effect increased cell adherence, but we did not address this possibility in our study.

In regard to the functional role of DEC-stimulated granulocyte adherence, previous in vitro studies of cell-to-parasite adherence have shown that DEC augments leukocyte adherence to microfilariae, particularly in the presence of immune serum. It has not been clear, however, how DEC brings about this interaction. Chandrasekaran et al. (3) reported that low-concentration DEC (5 μg/ml) enhances buffy coat adherence to microfilariae both in the presence and in the absence of immune serum. They also found that high concentrations of DEC (50 to 1,000 μg/ml) inhibit cell adherence and cell-mediated killing. In contrast, Piessens and Beldekas (14) found that DEC (5 to 100 μg/ml) promotes buffy coat adherence to microfilariae, but only in the presence of immune serum. Preincubation studies by this group indicated no direct DEC effect on cell adherence to microfilariae. Because of this, they concluded that the primary effect of DEC is on the parasite surface and not on the cell. Studies by Mackenzie, however, showed that feline granulocytes preincubated with DEC yield significantly greater adherence to untreated microfilariae (9). Presumably, the conclusions drawn by these independent investigators conflict because of differences in technique, including variations in cell, parasite, and serum sources used. The concentration-dependent effects of DEC and variations in experimental cell populations may account for the conflicting observations regarding the specific effects of DEC in these studies.

In experimental filariasis, DEC therapy induces rapid intravascular trapping of circulating microfilariae (7, 15, 18, 20, 21). Similarly, skin biopsy studies of patients with *Onchocerca volvulus* (a non-microfilaremic filarial infection) indicate that DEC causes peri-microfilarial inflammation within hours of treatment (5). These observations suggest that, in vivo, DEC acts, in part, by augmenting cellular inflammatory response in an infected host. This hypothesis is supported by our own data which indicate that DEC directly stimulates granulocyte adherence. Such a pharmacological mechanism represents an unusual and novel therapeutic effect.

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